Molecular Organization of Receptors

Efficacy, Agonists, and Antagonists[a]

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Intercellular and intracellular communication is of critical importance to the organization, differentiation, and coordination of all complex living systems. The need to maintain homeostasis and adjust to a changing environment is essential to the well-being of any organism. Complex living systems have adapted to their environments and developed highly differentiated cells with specific specialized functions by established communication networks that coordinate the structures and functions necessary for life. In general, the cells utilize a variety of chemical structures that serve as chemical messages to facilitate information transfer. These structures can be quite simple, such as, for example, glutamate, a simple amino acid, or alternatively they can be complex such as the wide variety of hormones and neurotransmitters of which insulin is an example. In general, the message is a signal for the receiving cell to modify or modulate its properties, and most commonly the messenger (hormone, neurotransmitter, growth factor, cytokin, etc.) manifests its effect by interaction with a cell surface receptor or acceptor molecule that generally is a macromolecule such as a protein or glycoprotein. This interaction generally leads to a change in the three-dimensional structure of the receptor. The conformational change in the receptor that accompanies formation of the ligand-receptor complex is the stimulus necessary to trigger a variety of chemical and physical events in the cell such as alternations in enzymatic activity, metabolism, ion channel properties, gene expression, and many other biochemical events. Interestingly, many hormones, neurotransmitters, and other messenger molecules are not highly selective and interact with a variety of receptor types and subtypes (TABLE 1). This promiscuity is also a useful tool for efficiency in the biological system, but presents difficulties in trying to understand the relationship(s) between the structure of a chemical messenger and its biological activity. The situation is further complicated by the fact that often a particular ligand which can interact with a well-defined receptor that has a particular function also can interact with other related receptors; indeed, this is often the case. Various types or subtypes of receptors have been postulated to exist, and in recent years these ideas have been proven by the cloning and expression of multiple receptor types and subtypes (TABLE 2). Although the examples in TABLES 1 and 2 are not comprehensive, they illustrate the complex way in which hormones, neurotrans-

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regulatory processes in the cell can be modified, and each will depend on complex kinetic and thermodynamic processes. At the moment, the complexity of what previously seemed like a rather "simple" pharmacological or physiological process appears to be increasing daily as new factors, enzymes, protein domains, and substrates increase. On the other hand, it seems clear that the cellular responses to ligand-receptor interactions as understood in terms of chemistry and structure open a new world of opportunity for understanding normal and disease processes and new possibilities for the treatment of disease.

Obviously, this is an exciting and rapidly expanding area of science, and one cannot possibly do justice to it in a short paper. The interested reader is referred to the literature and many excellent books on the subject[1–3] for a more comprehensive treatment. In this paper we briefly discuss the molecular organization of receptors from the standpoint of ligand-receptor interactions in particular, and then concentrate on the design of ligands for obtaining potent and selective agonists and antagonists, with particular emphasis on design considerations that lead to selectivity and high agonist and antagonist potency. Thus, we concentrate primarily on the ligands. Though many transmembrane receptors have been cloned and expressed and are available for functional studies, they still are not available in quantities sufficient for biophysical studies, and it appears that it will be some time before they will be available because of difficulties of purifying membrane-bound proteins and glycoproteins.

GENERAL CONSIDERATIONS

Efforts to understand the relationship between ligand structure and conformation and the dynamics and thermodynamics of the various binding events, as well as the biochemical changes they induce, are critical to future progress. This requires a highly interdisciplinary approach involving aspects of synthetic chemistry including asymmetric synthesis, computer-aided molecular design, biophysical studies of conformation and dynamics in the context of structure-activity relationships, and comprehensive multiple bioassay systems, so that aspects of potency, agonist/antagonist biological activity, and efficacy can be understood in terms of ligand structure, conformation, and topography. FIGURE 1 illustrates the interrelationships of each component of this comprehensive approach. If any component is left out it will be difficult to develop a successful approach.

Synthetic considerations are of primary importance because any effort to obtain highly potent and selective ligands will require a well-developed approach to synthesize designed ligands and then the ability to readily modify them for structure-activity studies, but also for studies of conformation and stability-bioavailability. Often these two latter kinds of studies require highly specialized amino acids, heterocyclics or other structures that contain specific isotopes or radioisotopes that need to be incorporated and that have minimal or no effects on biological properties. Furthermore, the current demands of science and medicine often require that the ligand of interest be prepared in a pure chiral state. This requires development of asymmetric synthesis or chiral resolution methods.

The design of ligands today generally is a combination of the classical medicinal chemistry approach of systematic modification of lead structures, and extensive use of biophysical studies (x-ray crystallography, nuclear magnetic resonance [NMR] spectroscopy, circular dichroism [CD] spectroscopy, fluorescence spectroscopy, and many other biophysical tools) in combination with computer-assisted modeling, molecular mechanics and quantum mechanics calculations, and molecular dynamics

studies. Since the major focus of our examples is peptide ligands for protein receptors, we consider the process from two levels, first, that of peptide structure and, second, that of peptide and peptidomimetic design. FIGURE 2 illustrates the principal conformational properties of the backbone of a polypeptide that is composed of ϕ, ψ, and ω torsion angles. The peptide bond is generally *trans* (180°) but also can be *cis* for X-proline and X-*N*-alkylated amino acid bonds. Thus the peptide backbone conformation is primarily a function of the ϕ and ψ angles, and Ramachandran[4,5] and co-workers showed many years ago that the low-energy secondary structures for peptides (α-helices, β-sheets, β-turns, extended conformations, etc.) could be defined by the ϕ and ψ angles and hence ϕ,ψ space often is referred to as Ramachandran space.[6] Given a particular peptide, careful analysis of ϕ,Ψ space can provide insights into the likely secondary structures for that peptide. This in turn can provide a starting point for further molecular design focused on enhancing some

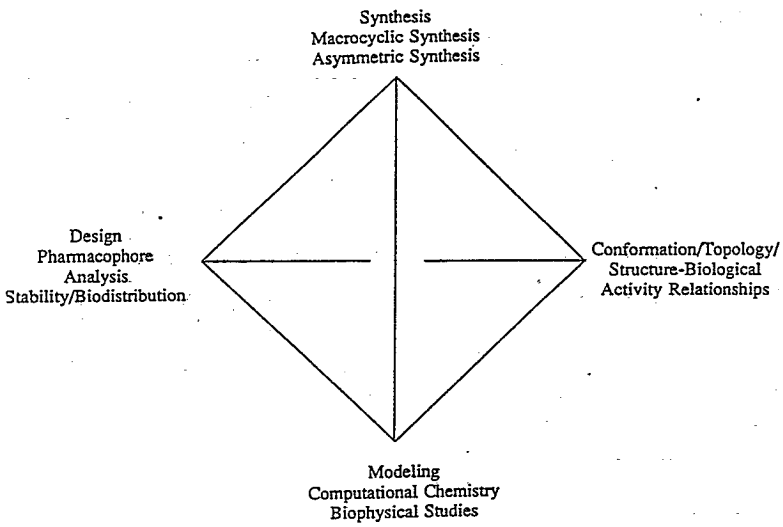

FIGURE 1. A comprehensive approach to receptor ligand design.

secondary structure and conformational property believed to be important for a particular biological effect.

In addition, one must carefully consider the side chain groups on each amino acid residue. Questions regarding their specific requirements for a particular receptor often can be addressed by an alanine scan or a glycine scan. In this approach, each amino acid residue is replaced one at a time either by Ala or by Gly, and the effect of each replacement examined in a binding assay or bioassay. For those compounds that remain highly potent, it is concluded that the side chain group of the particular residue substituted is not important for biological activity, whereas for those compounds that lose all activity, it is assumed to be very important. For those of intermediate biological activity, judgment is reserved. The major caveat for this approach is that if a large conformational change is induced by the substitution, the change in biological activity or potency may not properly reflect the actual situation.

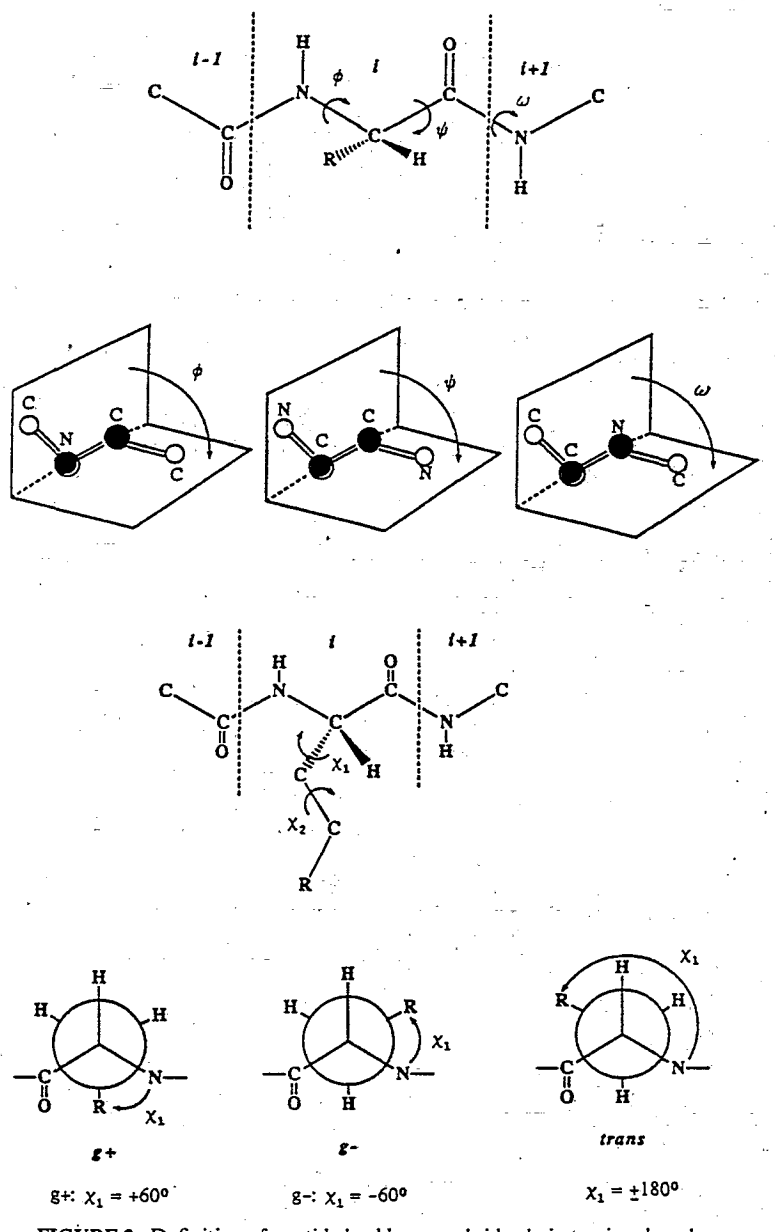

FIGURE 2. Definition of peptide backbone and side chain torsional angles.

Once specific side chains have been recognized as critical, the question arises as to which side chain conformation is important for a particular ligand-receptor interaction. FIGURE 2 defines the three main low-energy side chain conformations for most α-amino acids in a peptide: gauche(−) [g−], gauche(+) [g+], and *trans*, respectively. The chi angles are defined in FIGURE 2. Similar definitions can be given for x_2 and for further removed side chain moieties. Examination of side chain conformational space (often referred to as chi space) is of more recent origin (see, for example, refs. 7 and 8) and can be very illuminating. Recent studies have demonstrated that conformational constraints in chi space can have a profound effect on peptide and peptidomimetic biological potencies and selectivities.[7,9–12] The use of constraints in chi space is expected to play an increasingly important role in peptide and peptidomimetic design.

It is important to emphasize that the developments of the past 15 years have made it possible to develop a systematic approach to peptide and peptidomimetic design (TABLE 3).[12–17] The major goal of this approach is to define a specific

TABLE 3. Steps in Peptidomimetic Design

Define target
Establish multiple assay systems with positive and negative controls
Obtain peptide lead
Native ligand
Peptide libraries
Define key residues for molecular recognition and transduction (often different for agonists and antagonists)
Consensus sequence
Discontinuous epitope; address/message
Alanine scan; D-amino acid scan; etc.
Define pharmacophore
Local constraints
Global constraints–Built-in stability
Topographical constraints–Chi space
Agonist versus Antagonist
Selectivity
Efficacy
Design pseudopeptide, peptoid or nonpeptide scaffold
Surface and presentation platform
Optimize molecular recognition motif
Fine-tune for selectivity, potency, bioavailability, etc.

pharmacophore of a particular receptor or acceptor molecule, and evaluate its validity by specific design of a ligand with predictable agonist or antagonist activities.

It should be clear that ongoing evaluations of biological activities including potency, selectivity, efficacy, agonist activity, and antagonist activity are critical for the success of any ligand design process. Inasmuch as most native peptide ligands are not selective for one receptor type or subtype, it is critical to develop multiple bioassays and binding assays to be successful. There are now many examples of obtaining highly selective ligands for receptors from a nonselective lead structure, and this only is possible if multiple binding and bioassays are used. As for the assay, it should be remembered that a good assay is a chemical experiment in that valid results can be obtained only if the experiment is done at equilibrium and with full consideration of the laws of thermodynamics. The use of proper controls and the

elimination of nonspecific binding effects and nonspecific biological effects are also critical for success.

We now turn to a few specific illustrations of what is possible in the area of design of potent receptor selective ligands. We will emphasize studies from our laboratory with particular emphasis on ligands for the opioid receptors, especially the δ-opioid receptor type and its subtypes. First we will examine agonist activities, and then turn to antagonists.

POTENT AND SELECTIVE AGONISTS

Most naturally occurring hormones, neurotransmitters, growth factors, and other chemical messengers are agonists in interactions with their endogenous receptors, that is, they tend to induce or stimulate some specific activation response in the targeted cell. As previously discussed, most of these compounds do not have high selectivity for specific receptor types and subtypes related to their putative major site of biological activity. Thus, it often is difficult to sort out which biological effects are of primary physiological importance and which are of lesser importance. In view of these problems, it was necessary to develop much more selective ligands for specific receptors. A major hypothesis that we used to develop a systematic and rational approach to the problem is that each receptor type and subtype has specific and different stereostructural and conformational requirements for the ligands. To test this hypothesis requires the development of methods for introducing conformational and topographical constraints.[13–18] Often this approach has led to the development of highly potent and receptor-selective ligands. To illustrate this approach, we use the example of the development of constrained enkephalin and deltorphin/dermenkephalin analogues (TABLE 4).

In our initial approach with enkephalin we used pseudoisosteric cyclization[19] in which the side chain of the Met^5 residue in methionine enkephalin was substituted for a disulfide bridge that was attached to the α-carbon of Gly^2, and then to further constrain the cyclic 13-membered ring by use of geminal dimethyl groups on the β-carbons of both half-cysteine residues. This design led to the ligands of c[D-Pen^2, D-Pen^5]enkephalin (DPDPE) and c[D-Pen^2, L-Pen^5]enkephalin (DPLPE).[20] These cyclic peptidomimetic ligands of enkephalin were found to be highly potent ligands and to possess highly δ-opioid receptor selectivity because of their greatly reduced potency at μ-opioid receptors and their virtual inability to bind to κ-opioid receptors.[20,21] Comprehensive biophysical studies using two-dimensional NMR spectroscopy, molecular mechanics calculations, molecular dynamics simulations, and computer modeling[22,23] led to a proposed conformation in which the two aromatic residues were located on one lipophilic surface that was believed to be recognized by the δ-opioid receptor, but not by the μ- or κ-receptors. The question then arose as to what was the optimal topography of the aromatic side chain groups. This was particularly important because structure-activity studies[24] showed that both aromatic residues were important to the biological activity of these analogues. This has taken on added interest since the determination of the x-ray crystal structure of DPDPE,[25] which shows that the cyclic portion of the DPDPE structure in the crystal is very similar to that proposed in solution using NMR, but that significant differences exist in the conformation of the aromatic residues, especially of Tyr.

Stereoelectronic effects of the Phe^4 side chain of DPDPE were explored using a variety of parasubstituted groups.[26] Compounds with much higher potency and selectivity for the δ-opioid receptor were obtained, with the [p-BrPhe^4]DPDPE (TABLES 5 and 6) being especially selective and potent. We then explored chi space

TABLE 4. Structures of Naturally Occurring Delta Ligands

H-Tyr-Gly-Gly-Phe-Met-OH	Methionine enkephalin
H-Tyr-Gly-Gly-Phe-Leu-OH	Leucine enkephalin
H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2	Deltorphin I
H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2	Deltorphin II
H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2	Dermenkephalin

using a variety of constrained phenylalanine analogues such as 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic)[27] in which the chi-1 angle takes a preferred conformation of g(+). This compound was found to lose both its potency and selectivity for δ-opioid receptors (TABLES 5 and 6). On the other hand, when all four isomers of the constrained amino acid β-methylphenylalanine (β-MePhe) were placed in position 4 of DPDPE, a large differentiation of binding and biological activities (orders of magnitude in potency)[28] was observed. One of the compounds [the (S,S)-Phe[4]-containing compound], which has a preferred g(−) side chain conformation, was found to be the most potent and selective of the four isomers, and though it lost about 10-fold potency at the δ-receptor compared to DPDPE, it was about 10 times more selective in the binding assay (TABLES 5 and 6). In an effort to increase the lipophilicity of DPDPE for crossing the blood–brain barrier (BBB) and to develop a prohormone approach to DPDPE ligands (DPDPE is completely stable to biodegradation in the brain), we have found a remarkable new series of cyclic enkephalins, namely, compounds of the general structure H-Tyr-D-Pen-Gly-Phe(X)-Cys-Phe-OH ([Phe[6]]DPLCE) in which X = L, Cl, F, I, etc.[29] These compounds have higher affinity than do DPDPE for δ-opioid receptors, and the *p*-IPhe[4]-analogue is 1300-fold δ-receptor selective (TABLE 5). What is most remarkable is their potency in mouse vas deferens (MVD) bioassays for the δ-receptor where they are found to have potencies in the picomolar range. For example, [Phe[6]]DPLCE has an IC_{50} value of 16 picomolar and 5000-fold selectivity for the δ-receptor in the MVD assay (TABLE 6). These findings suggest that there are large differences in stereostructural requirements for the central and peripheral δ-opioid receptors.

TABLE 5. Binding Affinities and Selectivities of δ-Receptor Agonists

Compound	Potencies (nM)		Selectivities
	μ	δ	μ/δ
DPDPE	610	5.2	120
Deltorphin I	2,140	0.60	3,570
Dermenkephalin	1,900	0.47	4,000
H-Tyr-D-Pen-Gly-pBrPhe-D-Pen-OH	418	1.73	242
H-Tyr-D-Pen-Gly-Tic-D-Pen-OH	ND	ND	—
H-Tyr-D-Pen-Gly-(S,S) β-MePhe-D-Pen-OH	14,000	10	1,400
[Phe[6]]DPLCE	280	1.6	200
[pIPhe[4],Phe[6]]DPLCE	1,600	1.2	1,300
[(2S,3R) β-MePhe[3]]Deltorphin	> 72,000	2.5	> 29,000
[(2S,3R) β-MePhe[3]]Dermenkephalin	> 70,000	2.4	> 29,000
H-Tyr-D-Cys-Phe-Asp-Pen-Val-Gly-NH_2	3,760	2.2	1,700
H-Tyr-D-Pen-Phe-Asp-Pen-Nle-Gly-NH_2	55,000	4.8	11,500
[(S,S)-TMT[1]]DPDPE	720	210	3.5
[(S,S)-TMT[1]]Deltorphin	7,500	0.65	4,900

In the meantime, the first truly δ-opioid receptor selective natural ligands were found, the deltorphins and dermenkephalin (TABLE 4).[30,31] As seen in TABLES 5 and 6, these compounds were both more potent and more δ-opioid receptor selective than most of the designed cyclic enkephalin analogues. Given the different structures of these compounds and the cyclic enkephalins, the question arose as to why these compounds were so δ-opioid receptor selective, and what similarities and differences they might have with the cyclic enkephalins. A series of NMR studies in several laboratories showed that the deltorphins and dermenkephalin were highly flexible molecules with several possible low-energy conformations, and that these conformations were different from those of DPDPE. This led to the use of computation methods alone or in combination with NMR studies, to suggest possible topographical similarities that might account for their potent δ-opioid activities.[32–35] In the meantime, it was found that the lipophilic C-terminal tetrapeptide was critical for obtaining a potent and selective δ-ligand, because the N-terminal tripeptide (tetrapeptide) was in fact μ-opioid receptor selective (e.g., ref. 36). Thus, the C-terminal is

TABLE 6. Bioassay Potencies and Selectivities of δ-Receptor Agonists

Compound	Potencies		Selectivities
	GPI	MVD	
DPDPE	7,000	2.2	3,200
Deltorphin I	2,890	0.36	8,000
Dermenkephalin	3,400	0.28	12,000
H-Tyr-D-Pen-Gly-pBrPhe-D-Pen-OH	13,400	1.5	9,000
H-Tyr-D-Pen-Gly-Tic-D-Pen-OH	>300,000	1,500	>200
H-Tyr-D-Pen-Gly-(S,S) β-MePhe-D-Pen-OH	57,400	39	1,500
[Phe^6]DPLCE	83	0.016	5,200
[pIPhe^4, Phe^6]DPLCE	640	0.30	2,100
[(2S,3R) β-MePhe^3]Deltorphin	16,000	1.04	25,000
[(2S,3R) β-MePhe^3]Dermenkephalin	>100,000	1.75	>57,000
H-Tyr-D-Cys-Phe-Asp-Pen-Val-Gly-NH_2	1100	0.25	4,400
H-Tyr-D-Pen-Phe-Asp-Pen-Nle-Gly-NH_2	140,000	8.8	16,000
[(S,S)-TMT^1]DPDPE	290	170	1.7
[(S,S)-TMT^1]Deltorphin I	3,900	0.70	5,600

modulating the conformational properties of the N-terminal to give high δ receptor potency and selectivity. By use of β-methylphenylalanine in position 3, it was found that the selectivities and potencies in the binding assays and bioassays[37] were greatly dependent on the side chain conformation in ways that were different from those of cyclic enkephalin analogues (TABLES 5 and 6). Furthermore, large differences were seen for potencies at μ-opioid receptors (data not shown). These results led to the design of chimeras of the cyclic enkephalins and the linear deltorphins and dermenkephalin.[38] As expected (TABLE 5 and 6), these compounds were found to be highly potent and selective.

A most intriguing finding is that the efficacy of DPDPE and deltorphin/dermenkephalin analogues vary somewhat in the peripheral bioassays.[39] Generally, less than 10% occupancy of δ-receptors is sufficient for a full biological effect. From these studies, it is clear that much more effort should be placed on studies of those structural features that promote efficacy and more effective ways to measure efficacy. Finally, it should be noted that while these studies were in progress, DPDPE and

deltorphin, in functional antinociception assays, were found to be interacting with different δ-opioid receptors (see, e.g., refs. 40–45). By using δ-opioid receptor inhibitors, it was possible to block the antinoceception of one of the ligands while having no effect on the other. This led to the proposal for δ-receptor subtypes, δ_1 for DPDPE-related compounds, and δ_2 for deltorphin-related compounds. Interestingly, although clear-cut differences exist in the *in vivo* assays for δ_1 and δ_2 selective ligands, thus far no suitable radiobinding assay has been developed that can distinguish between them, even using radiolabeled δ_1 and δ_2 ligands. However, we recently have developed methods for the synthesis of tyrosine analogues such as β-methyl-2′,6′-dimethyltyrosine (TMT) (all four isomers). We have incorporated the (2S,3S) isomer into DPDPE and deltorphin I and have found the deltorphin I analogue (TABLES 5 and 6) to be highly potent and selective at δ_2-receptors, whereas the corresponding DPDPE analogue lost very significant binding and biological activity potency.[12] It has been demonstrated that these compounds still act at the δ_1- and δ_2-receptors, respectively. It is hoped that a good binding ligand can be developed from the deltorphin I analogue.

POTENT AND SELECTIVE ANTAGONISTS

In general, there is no straightforward way to develop antagonists of hormone and neurotransmitter ligands from knowledge of structure-activity relationships of agonists.[45] First, much evidence exists that agonists and antagonists bind differently to receptors (e.g., ref. 46) and thus, receptor selective antagonists must initially come from leads that are discovered in assays. Because binding per se cannot distinguish between agonists and antagonists, functional assays are required that measure either a second message or a specific bioactivity in tissue in whole animals. The reason agonists and antagonists have different structure-activity relationships is that they serve quite different functions on interacting with the receptor molecule (TABLE 7). In brief, because the conformation of a receptor in its agonist state is different from that in its antagonist or (inactive) state, it follows that the ligand-receptor interaction must be different to lead to a different conformation for the complex. We will discuss a few examples from our laboratory that illustrate a few of the principles of antagonist design. TABLE 8 lists some of the selective opioid receptor antagonists that are available.

An interesting example of antagonist design, based on a lead from a *different* receptor ligand, is the conversion of somatostatin (H-Ala-Gly-Cys-Lys-Asn-Phe-

TABLE 7. Agonist versus Antagonist Biological Activities

Agonist	Antagonist
Binds to a specific site in the receptor	Binds to the agonist site (competitive) or to some other site (noncompetitive) on the receptor
Leads to a change in the receptor	Need not lead to change in receptor conformation, but if it does it must be an inactive conformation
Often leads to phenomena such as patching, desensitization, etc.	Generally does not lead to patching, desensitization, etc.
Residence time on the receptor may be long or short	Generally requires long residence time on the receptor to be effective

TABLE 8. Selected Selective Opioid Receptor Antagonists

Ligand	Receptor Selective
D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP)	μ
D-Tic-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (TCTAP)	μ
[D-Ala^2, Leu^5, Cys^6]Enkephalin (DALCE)	δ_1
[Cys^4]Deltorphin I	δ_2
Naltrindole-5′-isothiocyanate (NTII)	δ_2
H-Tyr-Tic-Phe-Phe-NH_2 (TIPP)	δ
N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864)	δ
Naltrindole	δ
Naltrexone	μ
TENA	κ
Nor BNI	κ
β-Funaltrexamine (β-FNA)	μ
Naltriben (NTB)	δ_2
BNTX	δ_1

Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH) from a potent somatostatin receptor-specific ligand to a potent highly μ-opioid receptor specific antagonist. The approach came from three considerations.[47] First, it had been observed that at very high concentrations, somatostatin possessed analgesic activity. Second, the Merck group,[48] had determined that a β-turn around the Phe-Trp-Lys-Thr sequence (FIG. 3) could account for most of the potency at the somatostatin receptor. We felt this β-turn would poorly interact with the opioid receptor, and hence could be used as a scaffold (or template) on which to build an opioid ligand. Third, investigators at Sandoz[49] had found that their superpotent cyclic disulfide-containing octapeptide analogue of somatostatin also possessed some inhibiting activity in a neurotransmitter assay. Taking D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-OH as a lead compound, we found that substitution of the Phe^3 residue with Tyr, the Cys^7 residue with Pen and terminating the peptide as a carboxamide group, gave a compound D-Phe-Cys-Tyr-Phe-D-Trp-Lys-Thr-Pen-Thr- NH_2 (referred to as CTP) design that had binding properties completely different from somatostatin (TABLE 9). Unlike somatostatin which binds very poorly to μ- and δ-opioid receptors (TABLE 9), CTP binds strongly to the μ-opioid receptor, but only weakly to the δ-opioid and somatostatin receptors in the brain.[47] Subsequent studies showed that it was a potent *in vivo* μ-opioid receptor antagonist.[50] Comprehensive 2D NMR studies showed that CTP maintained the type II′ β-turn and provided considerable insight into the presentation to the receptor of the D-Phe^1, Cys^2, Tyr^3, Pen^7, and Thr-NH_2^{8} residues that appear to be involved in binding to the μ-opioid receptor.[51] Interestingly, although the μ-opioid receptor activity of CTP is that of an antagonist, CTP is an agonist at the δ-opioid receptor, but a very weak one.[52] Subsequent studies in which the highly constrained phenylalanine analogue, D-Tic was placed in position 1 gave an analogue, which was more potent and even more selective (TABLE 9),[52,53] and comprehensive 2D NMR and molecular dynamics investigations[54] were able to establish the preferred conformation and topology of [D-Tic^1]CTOP for recognizing the μ-opioid receptor in an antagonist state. Furthermore extensive *in vitro* and *in vivo* studies established that the [D-Tic^1]CTAP analogue showed none of the residual somatostatin-like activities that were found for CTP.[52,55] These compounds are now widely used as μ-opioid receptor antagonists, and are particularly useful for binding and *in vivo* studies

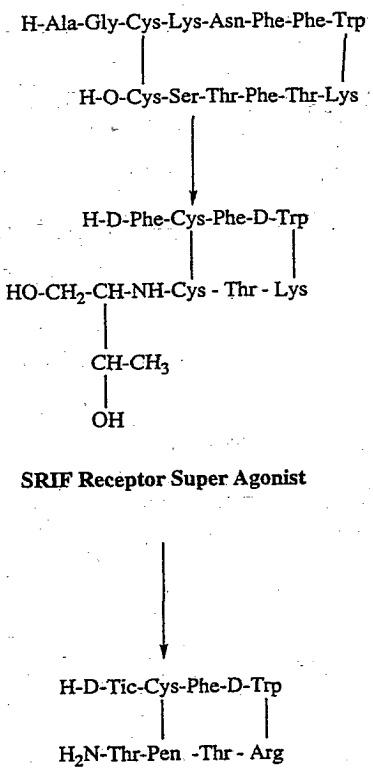

FIGURE 3. Conversion of somatostatin to a μ-opioid receptor antagonist. (Based on results from Bauer *et al.*[59]; Pelton *et al.*[47]; Kazmierski and Hruby.[7])

because they are completely stable to proteolytic breakdown and have a very prolonged *in vivo* inhibitory effect at μ-opioid receptors. Hence unlike naloxone, a single dose of compounds such as CTAP blocks *in vivo* μ-agonist activity such as analgesia for several hours.

A very interesting compound recently was discovered in our efforts to further investigate the topographical requirements for CTAP-related compounds. In this investigation, we place a cyclic D-tryptophan analogue D-tetrahydrocarboline (Tca) in position 1 of the cyclic octapeptide series. The analogue obtained, [D-Tca[1]]CTAP, had a most unusual activity profile.[56] Although it had only weak binding at μ- and δ-opioid receptors in the rat brain (IC_{50} values of 173 and 220 nM, respectively, TABLE 9), and has corresponding weak agonist activity in the MVD assay, it was a very weak partial agonist at μ-receptor in the GPI assay. However, it was a relatively

TABLE 9. Binding Properties of Selective μ-Opioid Receptor Selective Ligands

Compound	Binding Affinities (nM)			Selectivities	
	μ	δ	Somatostatin	δ/μ	Somatostatin/μ
Somatostatin	27,000	16,000	6	0.00038	.00022
CTP	3.7	8,400	3,690	2,300	1,000
[Orn^5]CTP (CTOP)	2.8	4,000	23,000	1,400	8,200
[D-Tic^1]CTAP	1.2	1,300	34,300	1,100	29,000
[D-Tic^1]CTOP	1.4	16,000	20,400	11,000	15,000
[D-Tca^1]CTAP	173	211	ND	1.2	—

potent analgesic (similar to DPDPE).[56] Subsequent studies have shown that the compound primarily interacts with the μ- and δ_2-receptors, and may be the first example of a compound that acts at the $\mu\delta_2$-receptor complex by "self potentiation."

A variety of other antagonists for opioid receptors have been developed in several laboratories, especially those of Portoghese[57] and Schiller.[58] The highly potent and δ-opioid receptor selective tetrapeptide of Schiller *et al.*,[58] H-Tyr-Tic-Phe-Phe-OH (TIPP), is particularly interesting, and demonstrates the power of conformation restriction in ligand design and its use in the discovery of antagonists. The finding that a single conformational change could covert a μ-opioid receptor agonist to a potent δ-opioid receptor antagonist is most intriguing.[58] All the antagonists in TABLES 8 and 9 and others are proving to be very useful in sorting out the complexities of the opioid receptor systems and in identifying the properties of the cloned receptors as they are isolated and expressed.

CONCLUSIONS

The complexity of most hormones and neurotransmitters as manifested by multiple ligands for a particular biological effect, and multiple receptor types and subtypes that have different biological activity profiles provide a profound challenge to chemists, biologists, and physicians who seek to understand these systems and translate that understanding into useful medicine. This complexity suggests that any understanding of these processes must incorporate an array of integrating systems. On the other hand, the unique biological profiles that appear to be associated with specific receptor types and subtypes suggest that highly selective ligands for a particular receptor can provide a highly specific effect and, it is hoped, useful medical treatments. The challenge to sort out and understand the complexities remains undiminished, but the opportunities it provides grow every day. It is hoped that chemists, biologists, biophysists, and medical doctors will cooperate and collaborate more frequently to accelerate this process.

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